Planar aggregation of the influenza viral fusion peptide alters membrane structure and promotes poration

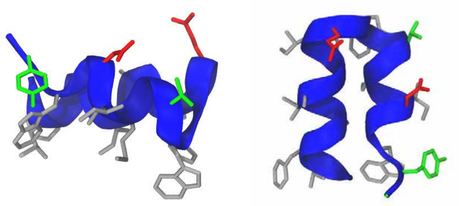
NIH National Heart, Lung, and Blood Institute

Richard W. Pastor, Amy Rice, Paul S. Blank, Joshua Zimmerberg

https://doi.org/10.17952/37EPS.2024.P2296

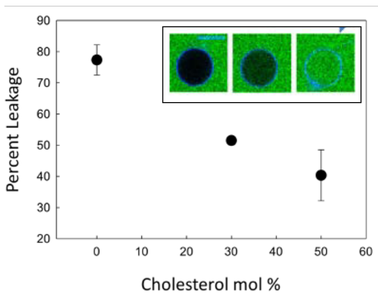
Background

The **fusion peptide** (FP) domain of influenza A hemagglutinin is the only portion of the virus that interacts directly with the target membrane of the host organism; its primary function is to facilitate fusion between the viral membrane and the host.



Side and top views of the helical hairpin structure[1] of Influenza A fusion peptide domain (sequence: **GLFGAIAGFIEGGWTGMIDGWYG***). Side chain coloring: red – anionic; green – polar; grey – nonpolar.*

Previous experiments and simulations have shown the ability of the isolated FP domain to aggregate when membrane-bound, and form pores in model membrane systems[2,3]; poration was demonstrated to be inhibited by cholesterol. It was hypothesized that FP clustering promotes poration by decreasing the hydrophobic thickness a pore must span in order to form.



FP-induced dye leakage in GUVs as a function of cholesterol content. The inset depicts a typical dye leakage experiment, where fluorescent dye leaks into the GUV after FP is added. Adapted from ref. [2].

However, in subsequent simulations cholesterol appeared to enhance FP clustering, seemingly in conflict with the original hypothesis. Here, we aim to resolve this apparent discrepancy by characterizing the energetic landscape of FP–FP interactions and elucidating the role that membrane-specific properties play in FP clustering and poration.

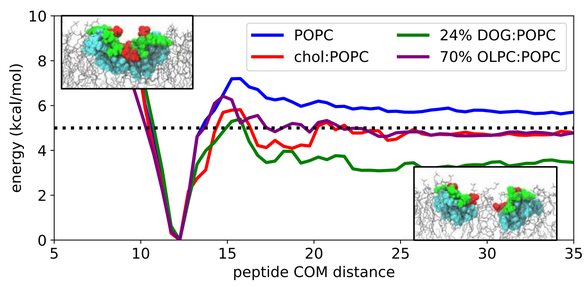
Simulation Details

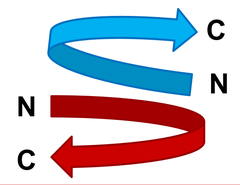
- CHARMM 36 force field
- Unbiased long timescale MD simulations
  - 10 FP in POPC, 1:1 chol:POPC, 7:3 OPC:POPC
    - 21 μs each
  - 21 FP in POPC and 1:1 chol:POPC
    - 3x10 μs each
  - 10 FP dimers in POPC and 1:1 chol:POPC
    - 3x10 μs each
- Free energy calculations – Umbrella Sampling
  - POPC, 1:1 chol:POPC, 7:3 OPC:POPC, & 24:76 DOG:POPC
  - Dimerization PMFs
    - 35 windows, 1 μs per window
  - Tetramerization PMFs
    - 26 windows, 1 μs per window
- Simulation packages
  - OpenMM [4] and Anton 2 [5]
- Analysis
  - PMFs – WHAM [6]1
  - RDFs – LOOS [7]

Results

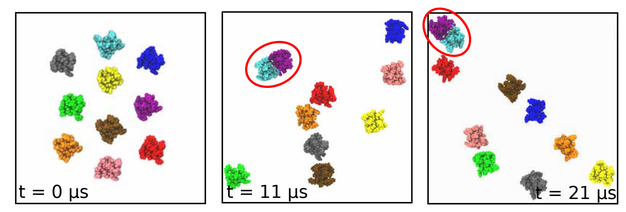
1 Dimers are favored regardless of bilayer constituents

***Potentials of mean force (PMF)**, for antiparallel dimer formation in four membrane compositions. The minima at a peptide COM distance of 12.2 Å corresponds to the antiparallel dimer structure.*



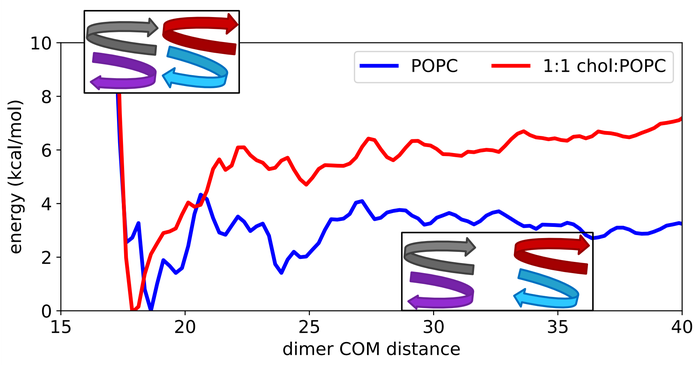


Antiparallel dimer stabilized, with ΔG ≈ −5 kcal/mol in the four membranes simulated, indicating population at equilibrium should primarily be antiparallel dimers



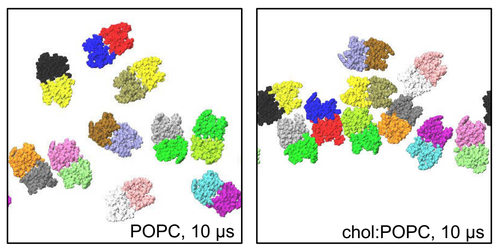
***Simulation snapshots**, depicting an antiparallel dimer (circled) that forms at t = 4 μs and remains stable through the remainder of the 21 μs simulation. Top down view of bilayer; lipids and water omitted. Taken from Ref [2]*

2 Tetramer formation is largely membrane-dependent



***Potentials of mean force (PMF)**, for tetramer formation from two antiparallel dimers. Both have a minima at a dimer COM distance ~17.7 Å, which corresponds to dimers with no lipid separating them. The PMF in **POPC has a second minimum at ~24 Å**, corresponding to separation by a single layer of lipids, a feature which is absent from the chol:POPC PMF.*

Dimer-dimer interfaces in POPC often include lipids, whereas in chol:POPC direct contact between dimers is more likely

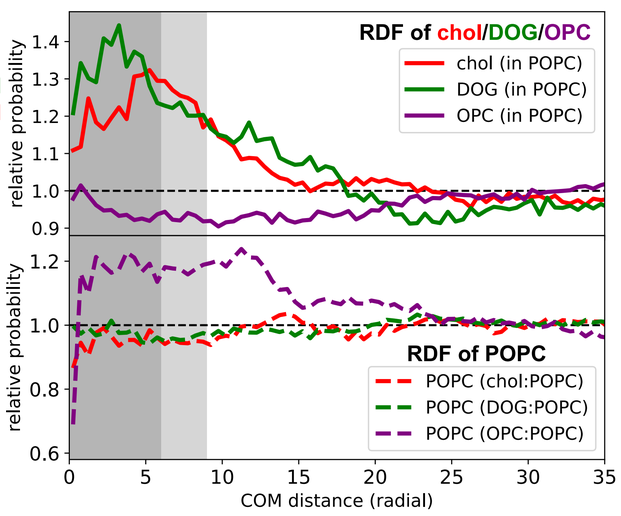


***Simulation snapshots**, depicting arrangement of ten antiparallel FP dimers in POPC (left) and chol:POPC (right) after 10 μs of equilibration. Top down view of bilayer, lipids and water omitted.*

3 Spontaneous curvature drives lipid enrichment in the bottom leaflet

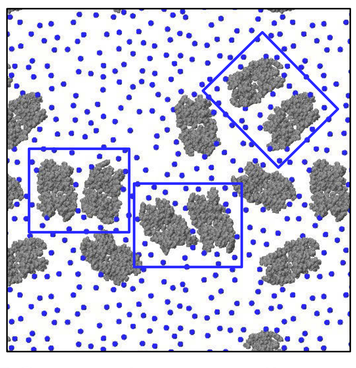
***Lipid radial distribution functions**, demonstrating lipid-dependent enrichment underneath an FP dimer. Lipids that generate negative spontaneous curvature (DOG and cholesterol) are enriched while lysolipid which generates positive spontaneous curvature is depleted. The gray shaded regions represent the approximate extent of one FP, along its shorter and longer axes.*

DOG – enriched
chol – enriched
OPC – depleted
POPC – enriched
POPC – depleted
POPC – depleted

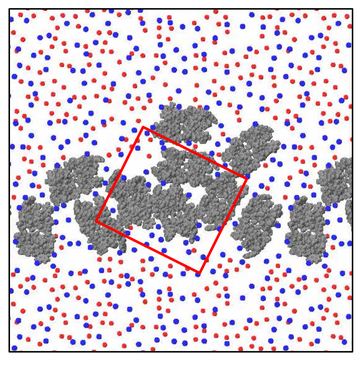


Lipids that generate negative spontaneous curvature are enriched under FP aggregates, while lipids that generate positive spontaneous curvature are depleted, consistent with predictions from theory [8]

Hypothesis



In pure **POPC** membranes, dimer-dimer interfaces typically include several lipids. These lipids are highly perturbed by the neighboring FP, destabilize the bilayer, and represent a weak point in the bilayer, thus acting as sites of pore nucleation.



In **chol**:**POPC** membranes, cholesterol is excluded from dimer-dimer interfaces and many interfaces contain no lipids. These direct peptide-peptide contacts prevent water from penetrating into the bilayer, potentially stabilizing the bilayer and inhibiting pore formation.

References

[1] Lorieau, Louis, Bax, *PNAS* **2010**, *107*, 11341–11346
[2] Rice et al. *Nat. Comm.* **2022**, *13*(1), 7336
[3] Rice, Zimmerberg, Pastor, *Biophys. J.* **2023**, *122*(6), 1018–1032
[4] Eastman et al. *PLOS Comp. Biol.* **2017**, *13*, e1005659
[5] Shaw et al. *IEEE* **2014**, 41–53
[6] Grossfield, "WHAM : the weighted histogram analysis method" v. 2.0.9
[7] Romo, Grossfield, *Conf. Proc. IEEE Eng. Med. Biol. Soc.* **2009**
[8] Barnoy, Parton, Kozlov, *bioRxiv*, doi: 10.1101/2024.07.09.602763
[9] Humphrey, Dalke, Schulten, *J. Molec. Graphics* **1996**, *14*, 33–38

Acknowledgements

This work was supported by the Intramural Program of the National Heart Lung and Blood Institute of the National Institutes of Health, Bethesda MD, USA, and utilized high performance computational resources at the National Institutes of Health (NHLBI LoBoS and HPC Biowulf clusters) and Anton 2 (PSC). Simulation snapshots rendered using VMD [9].

Conclusions

- Formation of antiparallel FP dimers is highly favorable, driven primarily by peptide-peptide contacts, and not strongly influenced by membrane composition
- Higher order clustering of FP is membrane dependent:
  - Cholesterol presence favors large aggregates with little lipid separating dimers
  - In POPC, interfaces separated by a single lipid layer are also favorable
- In all systems, POPC is enriched around FP
- Lipid composition under peptide aggregates is influenced by spontaneous curvature generation of lipids:
  - Lipids that generate negative spontaneous curvature are enriched
  - OPC (generates positive spontaneous curvature) is depleted
  - Enrichment of chol or DOG under FP may generate negative curvature, stabilizing tetramer formation
- Hypothesize that a single layer of lipids between adjacent peptides destabilizes the bilayer, facilitating poration – potentially explaining why cholesterol inhibits poration